Derivation and Properties of *Proteus mirabilis* Systems for High Frequency Transduction of Streptomycin- Sulphonamide and Streptomycin-Sulphonamide- Kanamycin Resistances

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SUMMARY

Properties of two transducing systems with phages capable of high frequency transduction (HFT) of streptomycin and sulphonamide resistance markers of the V group plasmid R905, and of these markers plus the kanamycin resistance marker derived from a previously described HFT phage 5006MHFTak, are described. Transducing particles of the former phage, named 5006MHFTsus, were detected using the replica-plate technique in an ultraviolet-induced lysate of *Proteus mirabilis* strain PM5006 transduced to streptomycin and sulphonamide resistance by phage 5006M grown on PM5006 carrying R905. Phage 5006MHFTsus was also detected by the replica-plate technique in ultraviolet-induced lysates of phage 5006MHFTsus transductants retransduced to ampicillin and kanamycin resistance by phage 5006MHFTak. Both phages were serologically identical to the parent phage 5006M. Ultraviolet-induced lysates transduced their markers to PM5006 at frequencies of about 5×10^{-2} /plaque-forming unit adsorbed for both the phages. With phage 5006MHFTsus, this frequency was increased about 10-fold by simultaneous infection of recipients with homologous non-transducing phage, while phage 5006MHFTsus transductions only underwent a twofold increase. Transductants took about 60 min to express complete resistance to 50 µg streptomycin/ml, and resistance to 1600 µg sulphadiazine/ml was complete within 120 min after phage adsorption. Phage 5006MHFTsus was slightly more resistant to ultraviolet inactivation of its transducing potential and reasons are given for the belief that transductants of both phages are heterogenote-like. Both phage lysates were also capable of generalized transduction and, like previously described HFT phages, lysates transduced the leucine marker at increased frequencies. Using previously described extra- and intra-species phage hosts, it was found that the phages could transduce in single infection and were defective in the lysogenic conversion function as well as in a maturation step. Possible modes of formation of the HFT particles are discussed.

INTRODUCTION

High frequency transducing (HFT) systems for the kanamycin and ampicillin resistance markers of plasmid R394 (Coetzee, Datta & Hedges, 1972) have been described in *Proteus mirabilis* (Coetzee, 1974b, 1975a), and for the kanamycin resistance marker of the same plasmid in a strain of Providence (Coetzee, 1975b). The phages involved were defective in the sense that transduction frequencies could be increased by simultaneous infection with homologous non-transducing phage. They also lacked immunity and possibly maturation functions, and in addition the *P. mirabilis* phages differed from their parent phage in that they failed to convert hosts to non-adsorption of homologous phage (Coetzee, 1976). The

tremendous selection offered by these antibiotic resistance HFT phages was used to obtain new hosts for the *P. mirabilis* phages (Coetzee, 1975*c*, 1976; see also Kondo & Mitsuhashi, 1966; Goldberg, Bender & Streicher, 1974; Mojica-A, 1975) which, due to very poor adsorption, would not have been discovered by other means. Use of these phages also proved that lysogenic conversion to homologous phage non-adsorption (Coetzee & Sacks, 1960; Coetzee, 1961, 1974*b*, 1975*a*) by phage 5006M, the parent phage (Krizsanovich, 1973), was not as absolute as had been thought (Coetzee, 1976; Coetzee & Krizsanovich-Williams, 1976).

During an investigation of transduction of markers of V plasmids (Hedges, 1975) by phage 5006M (Krizsanovich, 1973; Coetzee, 1975*a*, *c*, and unpublished results), the phage lysate of *P. mirabilis* PM5006(R905) transduced PM5006 to streptomycin and sulphonamide resistance at low frequency. The remaining marker of plasmid R905, chloramphenicol resistance, was not recovered and transductants could not transmit streptomycin or sulphonamide resistance by conjugation. This was reminiscent of the transductants which yielded the HFT phages mentioned previously. Because more phage variants are needed for development of this work, the streptomycin-sulphonamide resistant transductants were investigated for the possibility that they too might yield HFT lysates for the markers concerned.

METHODS

Bacteria, plasmid and phages. These are listed in Table 1.

Media. Nutrient broth was Oxoid no. 2, code CM67. Nutrient agar was the same broth solidified with 1.2 % (w/v) Difco agar. Wellcotest sensitivity test agar, code CM48, with added 0.15 % Difco bile salts no. 3 to inhibit swarming of *Proteus*, was used as the non-selective medium on which membranes with impinged bacteria were placed for development of phenotypic expression of resistance to sulphadiazine. The medium has a low content of sulphonamide antagonists. MacConkey agar was from Difco, and minimal medium was that of Grabow & Smit (1967). Incubation temperature was 37 °C.

Drugs and antibiotics. Ampicillin, kanamycin and nalidixic acid were used at 50 µg/ml. Rifampicin was used at 200 µg/ml. Streptomycin and sulphadiazine were used at 50 µg/ml and 200 µg/ml respectively unless otherwise stated. Sulphadiazine resistance of strains was tested by spotting saline-washed suspensions of overnight broth cultures on minimal medium containing various concentrations of the drug. The plates were examined for growth after 60 h incubation.

General phage techniques. These were described by Adams (1956) and Coetzee (1974*b*). Freeze-dried phage 34 antiserum (Coetzee & Sacks, 1960) with a neutralization constant (*K*) of 180 min⁻¹ against phage 5006MHFTk was used.

Conjugal transfer of plasmids and preparation of lytic phage lysates. Methods were as described by Coetzee *et al.* (1973).

Phage adsorption. The method used was described by Coetzee (1975*c*).

Ultraviolet irradiation of phage and phage induction. This was as described by Coetzee (1974*b*).

Concentration of phage lysates. The method described for P22 by Yamamoto & Alberts (1970) was used.

Replica screening technique for detection of HFT phage and transduction. This was done according to the filter technique of Coetzee (1974*b*) with the following modifications: the technique was used for transduction to prototrophy by filtering the adsorption mixture with a large excess of sterile saline before placing membranes with impinged cells on to minimal medium. For transductions to sulphadiazine resistance, the membranes, after filtering, were

Table 1. *Bacteria, plasmid and phages*

Bacteria	Properties*	Reference
<i>Proteus mirabilis</i> PM5006	Host for phages. Cryptically lysogenic for phage 5006M. Sensitive to C, S, Su	Coetzee & Smit (1970) Krizsanovich (1973)
PM5006 <i>arg-1</i> PM5006 <i>trp-1</i> PM5006 <i>leu-1</i> }	Auxotrophs of PM5006	Coetzee (1974 <i>b</i> , 1975 <i>a,c</i>) Coetzee (1974 <i>b</i>) Coetzee & Krizsanovich-Williams (1976)
PM5006 <i>str-r</i> PM5006 <i>nal-r</i> PM804	Spontaneous mutant resistant to 1 mg S/ml Spontaneous mutant resistant to 100 µg nalidixic acid/ml Cannot be shown to adsorb phages 5006M, 5006MHFT <i>k</i> , 5006MHFT <i>ak</i> but may be transduced to K and AK resistance by the last two phages respectively. Has a host-controlled modification system	Coetzee (1974 <i>b</i>) Coetzee (1974 <i>a</i>) Coetzee (1976)
PM804(AK) [†] N	Transductant of phage 5006MHFT <i>ak</i> Adsorbs above-mentioned phages well but restricts without modifying the phage	Coetzee (1976) Coetzee & Smit (1969)
NHI	Mutant of strain N which plates phage 5006M.PM5006 with an efficiency of 5×10^{-1}	Coetzee & Smit (1969)
<i>Proteus vulgaris</i> PV127	Adsorbs the above-mentioned phages very poorly. No plaque formation. Resistant to A. May be transduced to K resistance by both HFT phages	Coetzee (1975 <i>c</i>)
<i>Escherichia coli</i> J53-2	<i>Escherichia coli</i> K12 strain, auxotrophic for proline and methionine and resistant to rifampicin	Clowes & Hayes (1968)
Plasmid J53-2(R905)	J53-2 carrying the V group plasmid R905 which confers resistance to C, S, Su	Hedges (1975)
Bacteriophage 5006M.PM5006	Cryptic prophage of PM5006. Produced by lytic infection of PM5006. Generalized transducing for PM5006. Serologically identical to phages 34, 5006MHFT <i>k</i> and 5006MHFT <i>ak</i>	Krizsanovich (1973) Coetzee & Sacks (1960) Coetzee (1974 <i>b</i> , 1975 <i>a,c</i>)
5006MHFT <i>k</i> 5006MHFT <i>ak</i>	High frequency transducing for K resistance High frequency transducing for A, K resistance	Coetzee (1974 <i>b</i> , 1975 <i>c</i>) Coetzee (1975 <i>a, c</i>)
PM804 <i>ak</i>	Ultraviolet-induced HFT lysate of PM804(AK). Phage in the lysate bears the modification pattern of PM804	Coetzee (1976)

* A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphonamide.

† Superior line indicates that markers were transduced into the strain.

incubated on Wellcotest agar for 2 h, unless otherwise stated, before transfer to minimal medium containing sulphadiazine. Wellcotest agar was used in preference to nutrient agar as the carry-over of sulphonamide antagonists on the membrane from the latter medium interfered with the action of the sulphadiazine in the minimal medium. In all experiments involving minimal medium, plates were incubated for 60 h.

Selection of antibiotic-sensitive segregants. The method used was described by Coetzee (1975*c*).

RESULTS

Conjugal transfer of plasmid to PM5006 nal-r

In a conjugation experiment with selection for transipients on MacConkey agar containing nalidixic acid and chloramphenicol, J53-2(R905) transmitted the plasmid to PM5006 *nal-r* at a frequency of 2×10^{-5} /donor cell. Transipients were resistant to a maximum of

Table 2. *Transduction of markers of R factor R905 by phage 5006M to Proteus mirabilis strain PM5006*

Transductions were done as outlined in Methods. After the 10 min adsorption, the mixture was rapidly diluted into phage antiserum ($K = 40$) for 3 min, filtered and the membrane placed on agar which had just been spread with the same antiserum.

Lysate	M.o.i.	Recipient	Selected marker	Resistance of transductants*	Transduction frequency/p.f.u. adsorbed	Conjugal transfer of selected marker†
5006M.PM5006 <i>nal-r</i> (R905)	1.1	PM5006	S	SSu	1×10^{-9}	0/15
5006M.PM5006 <i>nal-r</i> (R905)	0.005	PM5006	Su	SSu	1×10^{-9}	0/12
5006M.PM5006 <i>nal-r</i> (R905)	1.1	PM5006	C	—	$< 2 \times 10^{-10}$	—
5006M.PM5006 <i>nal-r</i> (R905)	2.3	PM5006	C	—	$< 5 \times 10^{-10}$	—

M.o.i., multiplicity of infection; symbols for drugs as in Table 1.

* Saline suspensions of purified transductants were spotted on selective media to determine pattern of resistance.

† Recipient was J53-2; denominator refers to number of clones tested.

50 µg chloramphenicol/ml, 50 µg streptomycin/ml and 1600 µg sulphadiazine/ml and transmitted all these markers to J53-2 at about the same frequency, using rifampicin for contra-selection.

Preparation of phage 5006M.PM5006 nal-r(R905), transduction of R905 markers to PM5006 and properties of transductants

Phage 5006M lytically prepared on PM5006 *nal-r*(R905) had a titre of 2×10^{11} plaque-forming units (p.f.u.)/ml and transduced streptomycin and sulphonamide resistance to PM5006 at a frequency of 1×10^{-9} /p.f.u. adsorbed (Table 2). The chloramphenicol marker was never detected in transductants and transductants were not able to transmit streptomycin or sulphonamide resistance by conjugation. Seven of the transductant clones produced at very low multiplicity of infection (m.o.i.) of transducing phage, and reared in the presence of phage antiserum, were chosen for further study and were named PM5006($\overline{\text{SuS}}$)1 to 7. They were resistant to a maximum of 1600 µg sulphadiazine/ml and 50 µg streptomycin/ml and did not adsorb phage 5006M. Supernatants of overnight broth cultures of the seven clones yielded phage which plated on PM5006 to titres of about 1×10^5 p.f.u./ml. Replication of the phage titration plates to streptomycin agar yielded many colonies from the low dilution plates with phage from clones PM5006($\overline{\text{SuS}}$)5,7. The phage titration plates of the remaining five transductants did not yield growth on the replica plates. Based on past results (Coetzee, 1974*b*, 1975*b*), clone 5 was investigated for production of HFT phage.

Properties of phage induced from PM5006($\overline{\text{SuS}}$)5

Ultraviolet (u.v.) induction of PM5006($\overline{\text{SuS}}$)5 yielded a lysate with a titre of 6×10^7 p.f.u./ml on PM5006. The plaques were uniform in size and turbid. Numerous other inductions were attempted but the titre never exceeded 1×10^8 p.f.u./ml, and were in sharp contrast to phages 5006MHFT*k* and 5006MHFT*a**k* which could be obtained in titres nearly 100-fold higher (Coetzee, 1974*b*, 1975*a*). Neutralization tests with phage 34 antiserum revealed that the phage was neutralized at a frequency of 171 min^{-1} compared to 160 min^{-1} for phage 5006MHFT*k*. The phages were thus serologically identical. More than 99 % of plaque-forming phage in the lysate adsorbed to strains PM5006, N and NH1 within 12 min, but some adsorption (about 20 %) to PV127 could only be demonstrated on occasions, and adsorption to PM804 could not be shown (see Coetzee, 1975*c*, 1976). Results presented in

Table 3. *Transduction of P. mirabilis and a P. vulgaris strain by an ultraviolet-induced lysate of PM5006(SuS)5*

Transductions were done as outlined in Methods. Adsorption was for 10 min and membranes were incubated on non-selective media for 2 h before transfer to selective media.

M.o.i.	Recipient	Selected marker	Resistance of transductants	Transduction frequency/p.f.u. adsorbed or no. of transductants	Conjugal transfer of selected marker†
0.1	PM5006	Su	SSu	8×10^{-2}	0/10
0.01	PM5006	S	SSu	6×10^{-2}	0/7
0.8	N	Su	SSu	1×10^{-5}	0/4
0.1	NHI	S	SSu	5×10^{-4}	0/2
1.3*	PM804	Su	SSu	2	0/2
0.8*	PM804	S	SSu	4	0/4
0.7*	PV127	Su	SSu	$5 \times 10^{-8}\dagger$	0/4

For symbols, see Table 1.

* Multiplicity of infection was calculated using p.f.u. added; in all other cases, the calculation was based on p.f.u. adsorbed.

† Calculated as 20 % of input ratio.

‡ Recipient was J53-2.

Table 4. *Kinetics of phage 5006MHFTsus transduction and expression of streptomycin or sulphonamide resistance by PM5006 transductants*

Transduction conditions were as described in text. Time 0 coincided with the end of the adsorption period. Four and five membranes, respectively, were prepared for each experiment involving streptomycin and sulphadiazine. For the period indicated, membranes were kept on nutrient agar before transfer to streptomycin agar, or on Wellcotest agar before transfer to sulphadiazine minimal medium.

Incubation period (min)	No. of transductants/membrane on:								
	Streptomycin agar ($\mu\text{g/ml}$)				Sulphadiazine medium ($\mu\text{g/ml}$)				
	50	100	500	1000	100	200	400	800	1600
0	114	43	0	0	74	61	23	0	0
60	194	61	0	0	110	124	121	101	92
120	167	65	11	5	131	139	124	133	125
180	189	63	18	4	129	124	133	118	107

Table 3 indicate that the lysate transduced the two markers to PM5006 at a frequency of 8×10^{-2} /p.f.u. adsorbed. The transduction frequency to the restricting host N was more than 1000-fold lower, while that to NHI occurred at a frequency of about 5×10^{-4} /p.f.u. adsorbed. Some transductants were also observed with PM804 and PV127 as recipients. The HFT phage was named 5006MHFTsus. Complete phenotypic resistance to 50 μg streptomycin/ml was expressed in about 60 min and few transductants were resistant to more than 50 μg streptomycin/ml (Table 4). The level of resistance and phenotypic expression time were characteristic of plasmid rather than chromosomal resistance genes (Sugino & Hirota, 1962; Pearce & Meynell, 1968; Watanabe & Fukasawa, 1961; Coetzee & Sacks, 1960).

Expression of full resistance to 100 μg sulphadiazine/ml also took about 1 h (Table 4), and complete resistance to 1600 $\mu\text{g/ml}$ was achieved within 2 h. This was a surprisingly long period especially as a sulphonamide-resistant dihydropteroate synthase could be involved

Table 5. *Transduction of phage 5006MHFTsus transductants*

Transductions were done as outlined in Methods, and the legend to Table 3.

Lysate	Input ratio	Recipient	Selected marker	Resistance of transductants	No. of transductants	Conjugal transfer of selected marker*
5006MHFTak	1:2	PM5006(SuS)	K	AKSSu	24	0/5
5006MHFTak	1:2	PM5006(SuS)	A	AKSSu	31	0/4
5006MHFTak	1:3	PM804(SuS)	AK	AKSSu	4	0/4
5006MHFTak	2:6	PM804(SuS)	AK	AKSSu	8	0/3
PM804ak	1:1	PM804(SuS)	AK	AKSSu	26	0/3
PM804ak	2:2	PM804(SuS)	AK	AKSSu	31	0/4

Symbols for drugs as in Table 1.

* Recipient was J53-2.

(Skold, 1975). Results of this nature have not been published (see Kameda *et al.*, 1965; Watanabe, Furuse & Sakaizumi, 1968; Drabble & Stocker, 1968; Nakaya & Rownd, 1971). Abortive transductants were not found even in experiments where the period on non-selective media was eliminated (see Coetzee, 1975c).

Despite the use of low m.o.i., short adsorption periods and phage antiserum to prevent possible secondary infection on the membranes, transductants of PM5006 were invariably lysogenic in the sense that they spontaneously liberated phage active on PM5006. This was exactly what occurred with PM5006 transductants produced by phages 5006MHFTk and 5006MHFTak and could be due to complementation by the cryptic prophage (Coetzee, 1974b, 1975a). Despite the fact that all transductants were lysogenic and could not be shown to adsorb homologous phage, it was possible to transduce them to ampicillin and kanamycin resistance in small numbers with phage 5006MHFTak (Table 5, lines 1, 2). In contrast to PM804 transductants produced by phages 5006MHFTk and 5006MHFTak (Coetzee, 1976), all transductants of this organism by phage 5006MHFTsus were non-lysogenic. They did not liberate phage which plated on PM5006 either spontaneously or on u.v. induction, and transductants could be transduced (Table 5, lines 3, 4) to ampicillin and kanamycin resistance by homologous phage 5006MHFTak. The frequency of the latter transductions was increased once the phage bore the PM804 modification pattern (Table 5, lines 5, 6) (see Coetzee, 1976). Strains PVI27, N and NHI (not shown) also yielded non-lysogenic transductants at low multiplicities of phage 5006MHFTsus input. Strain NHI transductants adsorbed phage 5006MHFTsus as avidly as NHI but the phage did not form plaques on it. This may be taken as evidence for a defect in the lysogenic conversion function of the transducing phage since single infection of NHI by the parent phage 5006M was capable of converting it to homologous phage non-adsorption (Coetzee, 1976). Why the HFT lysate did not transduce strain NHI at frequencies similar to those at which it transduced PM5006 to antibiotic resistance is not known. It is unlikely that the difference could be ascribed to the small difference in efficiency of plating of the lysate on the two strains. None of the transductants mentioned above could transmit their resistance markers to J53-2 by conjugation (Tables 3 and 5).

Properties of transductants PM5006(SuS AK) and PM804(SuS AK)

The PM5006 and PM804 transductants bearing markers derived from serial transduction by phages 5006MHFTsus and 5006MHFTak (Table 5) were named PM5006(SuS AK) and

Table 6. Transduction of *P. mirabilis* and a *P. vulgaris* strain by an ultraviolet-induced lysate of PM5006(SuSK)

Transductions were done as outlined in Methods. Adsorption was for 10 min and membranes were incubated on non-selective media for 2 h before transfer to selective media.

M.o.i.	Recipient	Selected marker	Resistance of transductants	Transduction frequency/p.f.u. adsorbed or no. of transductants	Conjugal transfer of selected marker†
0.01	PM5006	K	SuSK	4×10^{-2}	0/5
0.01	PM5006	S	SuSK	4×10^{-2}	0/4
1.6*	PM5006(SuS)	K	SuSK	28	0/6
1.1*	PM804	K	SuSK	4	0/2
1.3	N	Su	SuSK	8×10^{-6}	0/2
0.3	NHI	Su	SuSK	5×10^{-4}	—
1.0*	PV127	Su	SuSK	$5 \times 10^{-8}\dagger$	0/2

For symbols, see Table 1.

* Multiplicity of infection was calculated using p.f.u. added; in all other cases, the calculation was based on p.f.u. adsorbed.

† Calculated as 20 % of input ratio.

‡ Recipient was J53-2.

PM804(SuS AK) respectively. Overnight broth cultures of PM804(SuS AK), or u.v. induction of these organisms, yielded low titre phage with properties of those derived from PM804(AK) (Coetzee, 1976). Phage capable of transducing sulphonamide or streptomycin resistance was not detected in these lysates by either replica plating or transduction techniques (see above). PM5006(SuS AK), on the other hand, yielded high titre phage on PM5006 following u.v. induction. Replication of the low-dilution titration plates to MacConkey agar which contained streptomycin, ampicillin and kanamycin yielded practically confluent growth and it was thought that a phage capable of transducing those three markers had been obtained. However, in these transductants (replica-plate transductants), the markers Su and S segregated independently from the A and K markers (results not shown).

Induction of many more independent clones of PM5006(SuS AK) and replication of the lysate's titration plates to MacConkey agar containing streptomycin plus ampicillin, or streptomycin plus kanamycin, led to the discovery of a lysate whose titration plates gave many transductants when replicated to streptomycin-kanamycin agar but none on the other agar. The source of the particular lysate was transductant clone PM5006(SuS AK)16. Further u.v.-induced lysates from the latter clone produced titration plates which replicated to both selective media, so recourse was made to the streptomycin-resistant replica-plate transductants mentioned above. Purified clones from these transductants were resistant to 50 µg streptomycin/ml, 100 µg kanamycin/ml and 1600 µg sulphadiazine/ml and were named PM5006(SuSK). On u.v. induction these transductants yielded lysates whose phage titration plates only replicated to streptomycin-kanamycin agar and not to media containing ampicillin. Using one of these lysates, PM5006 was transduced to sulphadiazine, streptomycin and kanamycin resistance at a frequency of 4×10^{-2} /p.f.u. adsorbed (Table 6). The low m.o.i. employed made it unlikely (but see Coetzee & Krizsanovich-Williams, 1976) that more than one phage was involved, and segregation experiments on these transductants (see below) confirmed that the markers were contained in single particles. The transducing phage in this lysate was named 5006MHFT_{susk}. Transductants were also obtained with PM5006(SuS) as recipient in numbers comparable to those obtained with phage 5006MHFT_{ak}

transductions to the same recipient (Tables 5 and 6). The lysate also transduced PM804, and strains N, NHI, and PV127 to sulphadiazine, streptomycin and kanamycin resistance in numbers comparable to those obtained with phage 5006MHFT_{sus} (Tables 5 and 6). Again no abortive transductants were detected. Strain PM804 transductants by this phage resembled those by phage 5006MHFT_{sus} in failing to liberate phage which plated on PM5006 (or PM804). Strain NHI transductants at low m.o.i. were capable of adsorbing homologous phage to the same extent as NHI but were not lysed by it. Like phage 5006MHFT_{sus}, phage 5006MHFT_{susk} may be regarded as defective in the lysogenic conversion function.

Segregation of markers from transductants

At least 10 % of clone-forming bacteria from overnight broth cultures of PM5006($\overline{\text{SuS}}$) or PM5006($\overline{\text{SuSK}}$) had segregated the transduced markers. Segregants of the former transductant which were sensitive to sulphadiazine were invariably also sensitive to streptomycin and *vice versa*. Similarly the segregants of the latter transductant sensitive to one of the antibacterial agents were also always sensitive to the other two drugs. The frequency of segregation was indicative of transduction by lysogenization and transductants were thus heterogenote-like (Luria, Adams & Ting, 1960; Coetzee, 1974*b*; but see Woods & Thomson, 1975). This conclusion was supported by observations of the effect that u.v. irradiation of the transducing lysates had on transduction frequencies (see below). The corresponding transductants of PM804 segregated at such a high frequency that it was sometimes difficult to align master and replicate plates correctly. The reason for the discrepancy in the segregation frequencies of the two organisms could be that the transducing prophage in PM5006 may sometimes (Coetzee, 1974*b*) be complemented by the resident cryptic prophage to mature and lyse the cell, whereas these PM804 transductants (but see Coetzee, 1976) were not found to be lysogenic for a cryptic prophage.

The effect of ultraviolet irradiation of HFT lysates

To confirm evidence presented above that transductions by the two HFT lysates proceeded by lysogenization, the lysates were irradiated for different periods of time. The curves relating time of irradiation to transduction frequencies and phage survival (Fig. 1) showed exponential declines, with the slopes of the former being about half that of the latter. This difference meant that some phage functions were not required for transduction (see Drexler & Kylberg, 1975). The exponential decline in transduction frequencies has been taken to mean that irradiation causes failure of the transduced element to persist as prophage (Luria *et al.*, 1960; Arber, 1960; Coetzee, 1974*b*). It is interesting that phage 5006MHFT_{susk} transductions were slightly more resistant to irradiation inactivation than those of phage 5006MHFT_{sus} which carries one less marker. These streptomycin-resistance transductions are in nice contrast to the u.v. inactivation of chromosomal streptomycin-resistance transductions (Coetzee & Sacks, 1960) and exclude a double crossover event between the HFT marker and the host chromosome (Luria *et al.*, 1960).

Effect of helper phage on transduction frequencies

Dilutions of each of the HFT lysates were made in constant concentrations of high-titre phage 5006M.PM5006 so that the m.o.i. of the latter phage in transduction mixtures with PM5006 was constant at about 3, 6 or 12 p.f.u. The m.o.i. of transducing phage in each of the three mixtures varied from 3.2 to 10^{-4} . With phage 5006MHFT_{susk}, the non-transducing

Fig. 1

Fig. 2

Fig. 1. The effect of u.v. irradiation on HFT lysates. Quantitative transduction experiments were done with the irradiated lysates at an m.o.i. of about 1 p.f.u. of the unirradiated phage. Selection was for streptomycin resistance. ○, Transducing activity of lysate 5006MHFTsus; □, transducing activity of lysate 5006MHFTsus; ●, plaque formation by both lysates on PM5006.

Fig. 2. Effect of helper phage on transduction frequency. Dilutions of the HFT lysates were made in a constant concentration of phage 5006M.PM5006. The m.o.i. of phage 5006M.PM5006 was about 3 p.f.u. ●, HFT lysate 5006MHFTsus alone; ○, with phage 5006M.PM5006. □, HFT lysate 5006MHFTsus alone; ■, with phage 5006M.PM5006.

Table 7. Transduction of PM5006 auxotrophs by lysates of high frequency transducing phages

Transductions were done as outlined in Methods. Because of the low titres obtained, the 5006MHFTsus lysate was concentrated more than 10-fold before use in these experiments. Adsorption mixtures were filtered with an excess of saline and membranes were placed directly on minimal medium for transductions to prototrophy. For transductions to antibiotic resistance, membranes were placed on nutrient agar for 2 h before transfer to selective media. The results of column 7 were obtained by replication of membranes on minimal medium to antibiotic agar.

Lysate	M.o.i.	Recipient	Transduction frequency/p.f.u. adsorbed			Prototrophy and S or SK
			Prototrophy	S	SK	
5006MHFTsus	1.1	PM5006 <i>arg-1</i>	9×10^{-8}	5×10^{-2}	—	$< 2 \times 10^{-9}$
5006MHFTsus	1.2	PM5006 <i>trp-1</i>	2×10^{-7}	5×10^{-2}	—	$< 2 \times 10^{-9}$
5006MHFTsus	1.1	PM5006 <i>leu-1</i>	5×10^{-3}	5×10^{-2}	—	5×10^{-6}
5006MHFTsus	0.001	PM5006 <i>leu-1</i>	5×10^{-3}	5×10^{-2}	—	$< 2 \times 10^{-9}$
5006MHFTsus	1.4	PM5006 <i>arg-1</i>	8×10^{-8}	—	5×10^{-2}	$< 5 \times 10^{-10}$
5006MHFTsus	0.9	PM5006 <i>trp-1</i>	5×10^{-8}	—	5×10^{-2}	$< 5 \times 10^{-10}$
5006MHFTsus	1.1	PM5006 <i>leu-1</i>	1×10^{-3}	—	5×10^{-2}	1×10^{-5}
5006MHFTsus	0.001	PM5006 <i>leu-1</i>	1×10^{-3}	—	5×10^{-2}	$< 5 \times 10^{-10}$

For symbols, see Table 1.

phage at an m.o.i. of 3, 6 or 12 raised the transduction frequency from 4×10^{-2} to 5×10^{-1} /p.f.u. adsorbed (Fig. 2). Non-transducing phage 5006M.PM5006 exerted its maximal effect on transduction by phage 5006MHFTsus at an m.o.i. of 3 but produced no more than a doubling in transduction frequency from 6×10^{-2} to 1.1×10^{-1} /p.f.u. adsorbed (Fig. 2). High frequency transduction by phage epsilon derivatives was not influenced by homologous non-transducing phage (Kameda *et al.*, 1965). The shapes of the curves relating m.o.i. of

transducing phage to transduction frequencies were similar for phages 5006MHFT_{sus} and 5006MHFT_{susk} and resembled those published (Coetzee, 1974*b*, 1975*b*, *c*). In particular, the rapid fall of the slopes between transducing phage m.o.i. of 1 and 10^{-1} was not indicative of mutual aid between transducing particles of a lysate (see Chan *et al.*, 1972).

Generalized transduction by HFT lysates

Both 5006MHFT_{sus} and 5006MHFT_{susk} lysates were capable of generalized transduction (Table 7). The leucine marker was transduced by both lysates at much higher frequencies than the other two markers due to the fact that the 5006M prophage normally integrates in close proximity to the leucine region of the host chromosome (Krizsanovich-Williams, 1975). Ultraviolet induction may then lead to faulty excision with encapsidation of leucine genes (Coetzee & Krizsanovich-Williams, 1976).

DISCUSSION

Phage 5006M has become associated with markers of two R factors – the T group plasmid R394 (Coetzee *et al.*, 1972) and R905, a V group plasmid (Hedges, 1975). However, the mechanism of formation of the HFT particles is not known. If the transducing particles could be shown to be integrated at the 5006M prophage site, it could be assumed that at least some of the original low frequency transducing particles for markers Su and S of R factor R905 contained portions of phage genome linked to these markers (Watanabe, 1971). The fact that PM5006 transductants produced at very low m.o.i. were lysogenically converted could mean that the gene for conversion had been reconstituted by integration of this element, containing at least some phage genes in association with drug-resistance markers, in tandem to the cryptic prophage (Krizsanovich, 1973; Coetzee, 1974*b*; Coetzee & Krizsanovich-Williams, 1976). The transducing elements themselves were defective in lysogenic conversion (see NHI transductants above) and it must be assumed that the genes concerned complemented (in some way) with those of the cryptic prophage of PM5006 to yield an active conversion product. Conversion to phage non-adsorption prevented the preparation of lytic generalized transducing phage to test for linkage of the antibiotic-resistance markers with the leucine marker (Krizsanovich-Williams, 1975). Linkage could not be demonstrated with phage in u.v.-induced lysates of transductants (Table 7; Coetzee & Krizsanovich-Williams, 1976) but this of course is not proof that the antibiotic-resistance markers were not integrated in the chromosome of transductants. The leucine marker does not fall within the chromosome linkage group which can be mobilized (Coetzee, 1975*d*), so interrupted mating experiments with antibiotic-sensitive leucine auxotrophs were not feasible. Working with a P22 system, Shipley & Olsen (1975) demonstrated that R factor RPI underwent transductional shortening to a size that carried only some of the resistance determinants but nevertheless was capable of maintaining itself extrachromosomally in transductants. The fact that transductants did not yield HFT phage was regarded as one of the properties which suggested that drug-resistance markers of RPI were not associated with a defective P22 prophage (but see Schwesinger & Novick, 1975). The transductants studied here produced HFT phage and this, taken in conjunction with the lysogenic conversion of PM5006 transductants, could mean that some genes of phage 5006M accompanied the antibiotic-resistance markers. A reasonable assumption is that the original low frequency transducing lysate contained phage in which some resistance determinants of plasmid R905 had replaced a segment of phage 5006M chromosome. A similar assumption was made by Kameda *et al.* (1965) to account for the phage epsilon HFT system. This is not

as unacceptable a suggestion as was formerly thought (Watanabe, 1971) for Schmieger (1970, 1971, 1972), Schmieger & Buch (1975) and Wall & Harriman (1974) have demonstrated that phages P22 and P1 do not simply play a passive 'phenotypic mixing' role in generalized transduction.

The retransductant PM5006($\overline{\text{SuS}}$ $\overline{\text{AK}}$)16 presumably contained both prophages as separate entities as it segregated the two sets of markers independently. But the ability of u.v.-induced lysates of this strain to transduce to PM5006 the capacity to yield HFT lysates of phage 5006MHFT susk suggests that early in the induction process, the kanamycin-resistance marker of the one particle recombined with the 5006MHFT sus vegetative phage to yield phage 5006MHFT susk (see Hoppe & Roth, 1974; Coetzee, 1975*b*). Experimental procedures did not allow for selection of the possible reciprocal recombinant, but phage 5006MHFT susk differs from phage 5006MHFT sus in its response to helper phage in the transduction process and in u.v. inactivation of transducing potential. This could mean that the recombination event was reciprocal and that phage 5006MHFT sus forfeited some portion of its genome. This is more likely than a transposition event (Hedges & Jacob, 1974) as the consequent increase in genome size could cause encapsidation problems (Chan *et al.*, 1972). An alternative explanation for formation of HFT phage is that transductional shortening (Shipley & Olsen, 1975) resulted in encapsidation of a fragment of the R factor R905 bearing the Su and S markers. This fragment could not maintain itself independently when injected by a phage capsid into recipient PM5006, and only at this stage did it recombine with the resident cryptic prophage. This is unlikely because, quite apart from the topological considerations, it is probable that some phage genetic material accompanied the R factor material since, even at very low m.o.i., transductants were lysogenically converted.

The fact that transductants of strain NH1 adsorbed homologous phage without plating it showed that the HFT phages studied here differed from phage lysates 5006MHFT k and 5006MHFT ak , which plated on transductants produced by homologous phage: phages 5006MHFT sus and 5006MHFT susk thus retained repressor activity. Due to poor adsorption and the m.o.i. used, transductants of PM804 may be assumed to have arisen through single infection. The very high segregation frequency of these transductants would certainly favour a heterogenote-like state and yet they did not liberate plaque-forming phage. This was indicative of a defect in maturation function, which phages 5006MHFT k and 5006MHFT ak did not possess in this organism (Coetzee, 1976). Transductants of phage epsilon were also defective lysogens (Kameda *et al.*, 1965). Infection with wild epsilon phage enabled these transductants to liberate HFT phage on occasions. It seems that superinfection by phage epsilon served a function which was performed by the cryptic prophage in PM5006.

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